potential use for this affinity labeling group.

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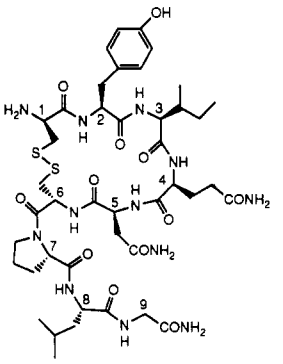
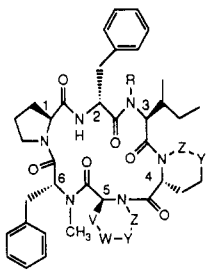
Receptor Ligands Which Bind the Oxytocin Receptor with Selectivity and High Affinity. Chemical Modification of a *Streptomyces silvensis* Derived Cyclic Hexapeptide

Oxytocin (OT) is a neurohypophyseal hormone which has been ascribed a pivotal role in parturition.[1] Evidence has accumulated to support the concept that the uterotonic action of OT and its stimulation of uterine prostaglandin release are events which combine to initiate labor.[2,3] Moreover, OT mediates the postpartum function of contracting the mammary myoepithelium to elicit milk letdown[4] and has also recently been implicated as a key element in preterm labor.[5,6]

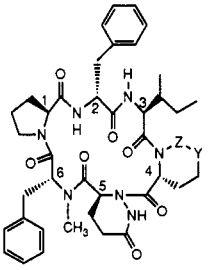
Since the breakthrough synthesis of OT three decades ago, considerable research has been devoted to the design of antagonists of this peptide hormone.[7,8] Interest in such compounds derives from the prospect of their use as novel therapeutic agents for the prevention of premature birth. While many promising in vivo antagonists of OT have been discovered over the years, the tactics for achieving this objective have generally been limited to modifications of the native OT and the closely related arginine vasopressin (AVP) structures.[3,5,7-14] One notable example is the development of the competitive OT antagonist 1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin.[15,16] Initial clinical evidence suggests that this peptide is efficacious in the inhibition of uterine contractions in premature labor in humans.[6,17]



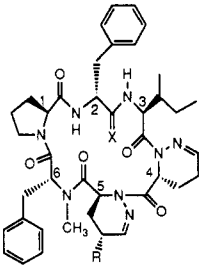
Oxytocin



(1) R = OH; V-W = CH_2-CH_2, Y-Z = CH_2-NH.
(2) R = H; V-W = CH_2-CH_2, Y-Z = CH_2-NH.
(3) R =H; V-W = CH_2-CH_2, Y-Z = CH=N.
(4) R = H; V-W = CH=CH, Y-Z = CH=N.
(6) R =H; V-W = CH_2-CH_2, Y-Z = CH_2-NCH_3.



(5) Y-Z = CH=N.
(7) Y-Z = CH_2-NH.



(8) X = O, R = $CH_2N(CH_3)_2$.
(9) X = S, R = H.
(10) X = H_2, R = H.

We recently reported the discovery of an entirely new structural class of OT antagonist, derived from a microbial source, and represented by **3**.[18] Despite the attractive OT/AVP antagonist properties of this compound (Table I), more potent and selective analogues with improved aqueous solubility[19] suitable for intravenous administration are required in order for compounds of this structural type to have practical utility. This communication represents our initial disclosure on chemoselective and regioselective transformations carried out on **3** with the aim of optimizing its physicochemical and pharmacological profile.

A key to the improved potency of **3** compared to its parent fermentation product **1** is oxidation of the piperazic acid (Piz) residues at positions 4 and 5. One hypothesis for this improved OT receptor affinity holds that changes in the conformation of these residues, brought about by the introduction of unsaturation, in turn influence the bioactive conformation of the 18-membered cycle. Ex-

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(19) The solubility of **3** in water at pH 6–8 is 68 μg/mL.

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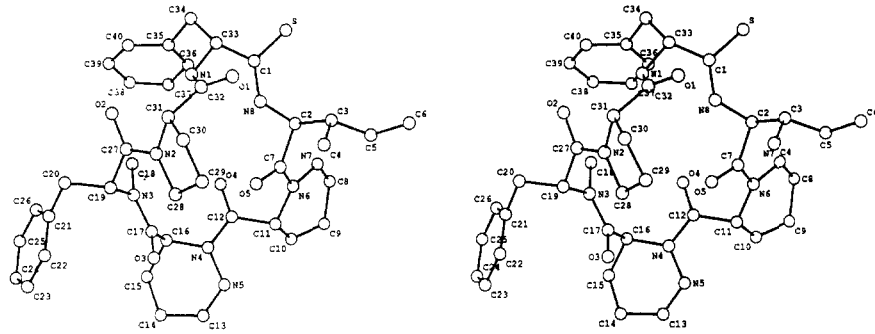

Figure 1. A computer-generated drawing of **9** from X-ray coordinates with hydrogens omitted for clarity.

Table I. Affinities of *Streptomyces silvensis* Derived Cyclic Hexapeptide 1 and Analogues for OT and AVP Receptors in the Rat

	K_i,[a] nM				
		[^{3}H]AVP		OT selectivity[b] vs	
compd	[^{3}H]OT (uterus)	V_1 (liver)	V_2 (kidney medulla)	V_1	V_2
OT	0.89	61 ± 7	88 ± 32	69	99
1	150 ± 23	2200 ± 260	3400 ± 420	15	23
2	30 ± 3.5	1500 ± 130	1500 ± 130	50	50
3	7.3 ± 0.58	730 ± 180	540 ± 30	100	74
4	8.1 (1)	390 (2)	580 (2)	48	72
5	9.6 ± 1.5	1600 (2)	6800 (2)	167	708
6	2500 (1)	7400 (1)	3100 (1)	3	1
7	89 (1)	3900 (1)	7800 (1)	44	88
8	3.4 ± 0.46	550 ± 83	1100 ± 84	162	324
9	1.1 ± 0.15	140 ± 28	70 ± 5.6	127	64
10	>30000 (1)	>30000 (1)	~30000 (1)		

[a] Details of the assay methodology are contained in ref 18. K_i values are the group mean ± SE for three to six determinations (unless otherwise noted in parentheses) and were determined[32] from the IC_{50} values generated using five to eight concentrations of compound in triplicate. [b] K_i[^{3}H]AVP/K_i [^{3}H]OT.

tensive ^{1}H NMR investigations of **3**[20] confirm that its conformation differs, at least in solution, from that of the X-ray crystal structure[21] obtained for **1**. In order to gauge the effect of the envelope (C_β-exo) conformation of the Piz residues on the conformation of the 18-membered cycle and correspondingly on the receptor-binding affinity of **3**, the latter compound was further oxidized. In this connection, a remarkable regioselectivity for reactions at L-Δ-Piz^5 versus D-Δ-Piz^4 was uncovered. For example, dehydrogenation of **3** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in hot toluene afforded the more planar vinylhydrazone **4** as the exclusive product.[22] This compound has affinity for the OT receptor comparable to that of **3** and displays modest differences in affinity for the AVP receptors. Alternatively, oxidation of **3** with alkaline hydrogen peroxide afforded only hydrazide **5**. Since the carbon atom adjacent to the Piz^5 β-nitrogen in both **3** and **5** is sp^2 hybridized, it may be assumed that the conformations of both modified Piz^5 residues are similar. The OT receptor binding affinities of **3** and **5** are in concert with this analysis. However, any loss in binding affinity of **5**, resulting from conformational differences vs **3**, may be compensated for by increased van der Waals interactions and/or hydrogen-bond donor–acceptor capabilities of the oxo-Piz^5 residue in **5** not available to Piz^5 in **3**. In this regard, it is noteworthy that reductive alkylation of the β-nitrogen of both Piz residues in **2** with methyl groups yielded compound **6**, which has 260–340-fold less affinity for the OT receptor than **5** and **3**, respectively. Interestingly, compound **2** could not be induced to react with iodomethane or dimethyl sulfate under a variety of conditions, reflecting the weak nucleophilicity of the Piz β-nitrogens. A substantial reduction in binding affinity for the OT and AVP receptors was obtained when the D-Δ-Piz^4 residue in **5** was reduced ($NaCNBH_3$) (cf. **7**). Again, it may be argued that conformation changes induced by the saturated D-Piz^4 residue and/or its modified hydrogen-bond donor–acceptor capabilities are responsible for this result. This and similar observations[23] lead to the conclusions that an unsaturated (envelope) D-Piz^4 residue is a potency-enhancing feature in this series of cyclic hexapeptides and that the L-Piz^5 residue is more amenable to functional group interchange, without loss in potency, than the D-Piz^4 residue. The prototypical example of the latter point is the chemoselective and stereoselective transformation of **3** to yield **8** (as a single isomer) on exposure to Eschenmoser's salt and camphorsulfonic acid in THF. The (dimethylamino)methyl derivative **8** is more potent than **3** and shows enhanced OT vs AVP selectivity. Morever, **8** (as its acetate salt) is more soluble in aqueous media than **3** (cf. 2.7 mg/mL vs 0.068 mg/mL at pH 7.3, respectively).

The consequence of changes in the peptide backbone of **3** was also explored. The introduction of pseudopeptide linkages in place of one or more amide bonds is a promising means of modifying peptide properties. To date, few peptide-bond replacements have been reported for cyclic peptides, and these have been prepared by total synthesis.[24] By exploiting the presence of only two secondary amides in **3**, we have succeeded in directly and selectively introducing pseudopeptide linkages into **3**. Reaction of **3** with the Lawesson reagent afforded thioamide **9**, a potent and selective OT receptor ligand. Its preparation was optimized (toluene, 90 °C, 1 h, slow addition of thionation reagent), thus providing it as the major reaction product (70% isolated yield, <10% thioamide byproducts).[25] Compound **9** was obtained as a crystalline solid whose structure was established by single-crystal X-ray diffraction analysis[26,27] and is displayed in stereoview in Figure 1. The 18-membered ring of **9** is markedly nonplanar due to a turn at the Pro^1 residue which appears to be stabilized by a hydrogen bond of approximately 2.9 Å between N1 and O2 (Figure 1 numbering). There is, however, no evidence for a classic β-turn. This backbone conformation

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results in the thioamide sulfur atom pointing away from the 18-membered ring while the phenyl ring of the adjacent Phe[2] residue is positioned over the central space of the macrocycle in close proximity to the methyl group of *N*-methyl Phe[6]. Interestingly, no upfield chemical shift for the *N*-methyl group of **9** was observed by ^{1}H NMR, relative to **3**, indicating that the positioning of the phenyl ring is likely a function of crystal packing.

The successful introduction into **3** of a single thioamide moiety afforded the opportunity of selectively obtaining the PheΨ[CH_2NH]Ile reduced peptide analogues. This modification has been useful for obtaining enzyme inhibitors[28] and hormone antagonists.[29,30] Further, it was anticipated that this change would augment the aqueous solubility of the products. Desulfurization of **9** with Raney nickel gave the expected **10**. In contrast to **9**, the OT and AVP receptor affinities of **10** were drastically reduced.

In sum, the selective chemical transformations carried out on the *Streptomyces silvensis* derived OT antagonist **3** have led to several analogues with improved OT receptor-binding potency, OT/AVP selectivity, and/or aqueous solubility. Compound **8**, the optimal example of these efforts, has been characterized as a functional OT antagonist similar to **3** in the blockade of OT-stimulated rat uterine contractions in vitro and in vivo.[18] Moreover, it shows no agonist activity in stimulating phosphatidyl inositol turnover in vitro or rat uterine contractions in vitro or in vivo. Details of these studies will be reported separately. Our results illustrate how subtle structural modifications can have dramatic effects on the ability of these compounds to bind to the oxytocin receptor, presumably through effects on conformation and/or hydrogen-bonding potential. These initial findings together with results obtained from totally synthetic analogues[31] have provided compounds of greater utility as research tools and have set the stage for the development of therapeutic agents.

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Azulene Derivatives: New Non-Prostanoid Thromboxane A_2 Receptor Antagonists

Numerous studies have demonstrated the potent vasoconstrictive and platelet-aggregative activities of thromboxane A_2 (TXA_2) in vivo[1,2] and in vitro.[3,4] TXA_2 has also been implicated in the etiology and pathology of many disorders such as coronary vasospasm,[5] myocardial ischemia,[6] asthma,[7] and peptic ulcer.[8,9] TXA_2/PGH_2 receptor antagonists may be more useful than TXA_2 synthetase inhibitors, because these compounds also antagonize the effects of endoperoxides and do not lead to accumulation of endoperoxide intermediates.[10] On the other hand, TXA_2 synthetase inhibitors are less effective in a disease in which TXA_2 may be continuously produced.[10] Recently, we found that an azulene derivative, 3-Ethyl-7-isopropyl-1-azulenesulfonic acid sodium salt-0.33 hydrate (KT1-32), showed a mild TXA_2 antagonistic activity.[11] Therefore, we synthesized various azulene derivatives in order to evaluate their TXA_2 antagonistic activity in two major TXA_2/PGH_2 receptor subtypes, α-receptor for platelet aggregation and τ-receptor for vascular contraction.[12] In this study, selectivity of compounds for one or the other receptor subtype is examined.

The target compounds were synthesized according to the routes shown in Scheme I. Condensation of 6-Isopropyl-3-(methoxycarbonyl)-2*H*-cyclohepta[*b*]furan-2-one (**1**) with morpholino enamines of aldehydes (n = 3–5),[13,14] followed by cleavage of the benzyl ether, resulted in alcohols **3**. Tosylation of **3** and subsequent displacement of tosylates with sodium salts of benzenesulfonamides afforded N-substituted sulfonamides **4**. Hydrolysis of **4** furnished carboxylic acids **5** (Scheme I). Sulfonic acid sodium salt derivatives **7** were prepared from **4** according to the method reported previously.[13] The synthesis of **10** started with **6** by using Anderson's stepwise construction of the acetic acid homologue.[15] α,β-Unsaturated carboxylic acid derivatives **13** were prepared by the Vilsmeier–Haack formylation of **6** followed by Horner–Emmons reaction[16]

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